claims priority from a Canadian patent application number 2,282,179, filed September 7, 1999 and U.S. application number 60/163,546, filed November 4, 1999. - -

At page 48 between the first paragraph and the "Example 1..." please insert the following:

-- The invention also teaches a library as described herein, wherein said parental ligand binding molecule is built on a V_H framework which is at least 80% homologous (preferably 85% homologous, more preferably at least 90% homologous) to the framework regions and conserved regions of a human V_H domain derived from a IgM.

The invention also teaches a library as described above, wherein said parental ligand binding molecule is encoded by a nucleic acid sequence comprising nucleic acid residues 6-48 as shown in Figure 3.

The invention also teaches a library as described above, wherein said parental ligand binding molecule is encoded by the nucleic acid sequence depicted in Figures 3 or 4.

The invention also teaches a library as described above, wherein said parental ligand binding molecule comprises at least in substantial part the FR2 region of the immunoglobulin V_H domain depicted in Figure 1, including amino acid residues 44, 45 and 47.

The invention also teaches a library as described above, wherein one or more residues selected from residues 4 to 21 in FR1 are partially randomized.

The invention also teaches a library as described above, wherein one or more residues selected from residues 100o to 114 in FR4 are partially randomized.

The invention also teaches a library as described above, wherein the residues selected for partial randomization 100o to 114 in FR4 are randomized at least 75% and preferably 90% in favour of the residues depicted in Figure 1.

The invention also teaches a library as described above which is a phage display library.

The invention also teaches a phage display library displaying a plurality of different variants of a parental ligand binding molecule, wherein said parental ligand binding molecule comprises an immunoglobulin V_H binding fragment comprising, at least in substantial part, at least the FR regions of the immunoglobulin V_H fragment depicted in one of Figures 1 or 2 and wherein said variants are encoded by nucleic acid sequences which vary from the nucleic acid sequence encoding said parental ligand binding molecule in a subsequence encoding at least part of one of the CDRs of said parental ligand binding molecule, whereby said plurality of variants comprise at least in substantial part, the FR regions of the immunoglobulin V_H fragment depicted in such Figure 1 or 2 and are differentiated, at least in part, by amino acid variations encoded by variations in said subsequence.

The invention also teaches a heterogeneous population of genetic packages (eg. phage) having a genetically determined outer surface protein, wherein the genetic packages collectively display a plurality of different, preferably human, (ie. substantial identity to human) V_H ligand-binding fragments, each genetic package including a nucleic acid construct coding for a fusion protein which comprises at least a portion of the outer surface protein and a variant of at least one soluble parental ligand-binding fragment preferably derived from or having a substantial part of the FR regions of the amino acid sequence identified in one of Figures 1 or 2, (or a sequence at least 80%, preferably 85 to 100%, more preferably 90-100%, homologous (ie. identity) thereto), wherein the V_H binding-fragment preferably spans from a position upstream of an immunoglobulin heavy chain CDR1 to a position downstream of CDR3 (preferably including substantially all of FR1 and/or FR4), and wherein at least part of a CDR, preferably the CDR3, is a randomly generated variant of a CDR of said parental V_H ligand binding-fragment and wherein the fusion protein is preferably expressed in the absence of an immunoglobulin light chain whereby the potential V_H binding fragments are, on the whole, better adapted to be or better capable of being expressed as soluble proteins.

The invention also teaches a ligand binding molecule which is a variant of a parental ligand binding molecule which comprises an immunoglobulin V_H binding domain, said parental binding molecule comprising, at least in substantial part, at least the FR regions of the immunoglobulin V_H fragment depicted in one of Figures 1 or 2 and wherein said variant comprises, at least in substantial part, the FR regions of the immunoglobulin V_H fragment depicted in the corresponding such Figure 1 or 2 and differs from said parental ligand binding molecule at amino acid residues constituting at least part of one of at least one of the CDRs of said parental ligand binding molecule.

The invention also teaches a ligand binding molecule which is derived from a variant of a parental ligand binding molecule which comprises an immunoglobulin V_H binding domain, said parental binding molecule comprising, at least in substantial part, at least the FR regions of the immunoglobulin V_H domain depicted in one of Figures 1 or 2 and wherein said variant comprises, at least in substantial part, the FR regions of the immunoglobulin V_H fragment depicted in the corresponding such Figure and differs from said parental ligand binding molecule at amino acid residues constituting part of one of the CDRs of said parental ligand binding molecule.

The invention also teaches a ligand binding molecule which has been identified as binding to a target ligand by screening a library as described herein for one or more ligand binding molecules which specifically recognize said target ligand.

The invention also teaches a combinatorial library comprising variants of a parental ligand binding molecule, wherein said parental ligand binding molecule comprises an immunoglobulin V_H fragment comprising at least in substantial part, at least the FR regions of the immunoglobulin V_H domain depicted in Figure 1 and wherein said variants comprise, at least in substantial part, at least the FR regions of the immunoglobulin V_H domain depicted in Figure 1 and differ from said parental ligand binding molecule at amino acid residues constituting part of at least one of the CDRs of said parental ligand binding molecule.

The invention also teaches a library as described above, wherein at least a substantial number of said variants comprise at least one of the following mutations: G44E, L45R, Y47G, V93A, K94A.

The invention also teaches a library as described above, wherein at least a substantial number of said variants comprise the following mutations: G44E, L45R, Y47G.

The invention also teaches a library as described above, wherein at a substantial number of said variants comprise the following mutations: G44E, L45R, Y47G, V93A, K94A.

The invention also teaches a library comprising a heterogeneous population of genetic packages which collectively display a plurality of different potential V_H binding fragments, each said genetic package having:

(a) an outer surface having an outer surface protein; and

(b) a nucleic acid construct coding for a fusion protein, said fusion protein including:

(i) at least a portion of said outer surface protein; and

(ii) a V_H binding-fragment spanning from a position upstream of an immunoglobulin heavy chain CDR1 to a position downstream of CDR3, wherein at least part of said CDR3 is a randomly generated variant of a CDR3 of a non-camelid or a non-camelid type parental V_H binding-fragment; and

wherein said fusion proteins are expressed in the absence of an immunoglobulin light chain protein or portions thereof on said outer surface of said genetic packages, and wherein said potential V_H binding fragments are adapted to be or capable of being expressed as soluble proteins.

The invention also teaches a library as described above, wherein said potential V_H binding fragments have a CDR3 length of 16 to 33 amino acids.

The invention also teaches a library as described above, wherein said V_H binding-fragment comprises fragments FR1 to FR4.

The invention also teaches a library as described above, wherein each said genetic packages is a phage and said library is a phage display library.

The invention also teaches a library as described above, wherein said V_H binding fragments comprise fragments FR1 to FR4.

The invention also teaches a library as described above, wherein CDR3s of a variety of different lengths from 16 to 33 amino acids are predominantly represented in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein CDR3s of a variety of different lengths from 17 to 23 amino acids are predominantly represented in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein CDR3s of 23 amino acids in length are predominantly represented in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein said potential V_H binding-fragment is built on a V_H framework which is at least 80% homologous to the framework regions of human V_H.

The invention also teaches a library as described above, wherein said parental V_H binding-fragment is derived from a human V_H chain identified in Figure 1 or is built on any framework which is at least 80%homologous to the framework and other conserved regions of said human V_H chain.

The invention also teaches a library as described above, wherein said parental V_H binding-fragment is adapted or adaptable to a human framework.

The invention also teaches a library as described above, wherein the amino acids in one or more series of CDR3 amino acids selected from the groups of amino acids consisting of 95-100 and 100i-100n are preserved in approximately at least 90% or approximately 100% of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein one or more amino acids in one or more series of CDR3 amino acids selected from the groups of amino acids consisting of 95-100, 100i-100n, 100o-102 and 101-102 of Figure 4are preserved, on an amino acid by amino acid basis, in approximately at least 90% or approximately 100% of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein said potential V_H binding fragments have a native human V_L interface at positions 44, 45, and 47 of Figure 1.

The invention also teaches a library as described above, wherein said potential V_H binding fragments have non-hydrophobic amino acids at least one of positions 44, 45, and 47 of Figure 1.

The invention also teaches a library as described above, wherein said potential V_H binding fragments are further characterized by a CDR3 containing an amino acid sequence which is at least 90% homologous to at least one region of conserved amino acids selected from those regions identified in Figure 1.

The invention also teaches a library as described above, wherein said potential V_H binding fragments are furthe r characterized in that at least approximately 50% of the amino acids corresponding to the amino acids at positions 100a-100h shown in Figure 1 are biased in favor of wild-type A6 to produce at least 10% wild-type amino acid at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein said potential V_H binding fragments are furthe r characterized in that at least approximately 90% of the

amino acids corresponding to the amino acids at positions 100a-100h shown in Figure 1 are each 10% biased in favor of wild-type A6 to produce at least 10% wild-type amino acid at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein one or more individual amino acids in positions 100a-100b and 100g-100h, or 100a-100c and 100f-100h, are wild-type in at least approximately 10% of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein individual amino acids in positions 100a-100b and 100g-100h, or 100a–100c and 100f-100h, are wild-type in at least approximately 50% of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least 50% of individual amino acids in positions 95-100 are biased in favor of wild type to produce at least 10% wild-type amino acid at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least 90% of the individual amino acids in positions 95-100 of Figure 1 are biased in favor of wild-type to produce at least 10% wild-type amino acid at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least 50% of the individual amino acids in positions 100*i* - 100*n* in Figure 1 are biased in favor of wild-type to produce at least 10% wild-type amino acid at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least 50% of the individual amino acids in positions 100*i* - 100*n* in Figure 1 are biased in favor of wild-type to produce at least 50% wild-type amino acid at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein individual amino acids in any one or more of positions 100a-100b, 100g-100h, 100l and 100o are biased to produce at least 10% of wild-type amino acids, aromatic amino acids or amino acids selected exclusively from the group consisting of tyrosine, histidine, glutamine, asparagine, lysine, aspartic acid and glutamic acid, wild-type amino acid at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein amino acids in any one or more of positions 100a-100b, 100g-100h, 100l and 100o are biased to produce at least 50% of wild-type amino acids, aromatic amino acids or amino acids selected exclusively from the group consisting of tyrosine, histidine, glutamine, asparagine, lysine, aspartic acid and glutamic acid, at said positions in said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least 5 consecutive amino acid positions among positions 95-100n shown in Figure 1 are biased to produce at least 10% wild-type amino acids at said positions of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least 8 consecutive amino acids positions among residues 95-100n shown in Figure 1 are biased to produce at least 10% wild-type amino acids at said positions of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least 10 consecutive amino acids among residues 95-100n shown in Figure 1 are biased to produce at least 10% wild-type amino acids at said positions of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least amino acids positions 100a-100b to 100f-100h and 100m are biased to produce at least 50% wild-type amino acids at said positions of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least amino acids positions 100f to 100m are biased to produce at least 50% wild-type amino acids at said positions of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein at least amino acids positions 100o to 102 or 101 to 102 are biased to produce at least 10% wild-type amino acids at said positions of said potential V_H binding fragments.

The invention also teaches a library as described above, wherein framework regions are at least approximately 90% homologous to that of the wild-type parental binding-fragment shown in Figure 1.

The invention also teaches a library as described above, wherein the CDR2 region is at least approximately 80% homologous to that of the wild-type parental binding-fragment shown in Figure 1.

The invention also teaches a library as described above, wherein the CDR1 region is at least approximately 80% homologous to that of the wild-type parental binding-fragment shown in Figure 1.

The invention also teaches a library as described above, wherein the CDR1 region is biased to have a cysteine residue for forming a loop in said V_H binding fragment by means of interaction of said cysteine with a randomly generated cysteine residue in CDR3.

The invention also teaches a library as described above, wherein said recombinant phage are constructed in an M-13 derived vector and said phage coat protein is pIII.

The invention also teaches a library comprising a heterogeneous population of genetic packages which collectively display a plurality of different potential binding fragments, each said genetic package having:

(a) an outer surface having an outer surface protein; and

(b) a nucleic acid construct coding for a fusion protein, said fusion protein including:

(i) at least a portion of said outer surface protein; and

(ii) a randomly generated variant of a non-camelid or a non-camelid type parental binding fragment;

wherein at least a part of said construct is biased in favor of producing said fusion proteins which are expressed as soluble proteins.

The invention also teaches a library comprising a heterogeneous population of genetic packages which collectively display a plurality of different potential binding fragments, each said genetic package having:

(a) an outer surface having an outer surface protein; and

(b) a nucleic acid construct coding for a fusion protein, said fusion protein including:

(i) at least a portion of said outer surface protein; and

(ii) a randomly generated variant of a non-camelid or a non-camelid type parental binding fragment;

wherein at least a part of said construct is biased in favor of producing said fusion proteins having the amino acid construct of said parental binding fragment.

The invention also teaches a library as described above, wherein said construct is biased in favor of producing soluble fusion proteins.

The invention also teaches a library as described above, wherein said parental binding fragment is a V_H binding fragment, and said construct either includes at least a portion of amino acids 95 to 100o of Figure 1.

The invention also teaches a library as described above, wherein said parental binding fragment is a V_H binding fragment, and said construct either includes at least a portion of amino acids of CDR3.

The invention also teaches a library as described above, wherein said genetic package is a phage and said soluble parental binding-fragment is selected from the group consisting of an scFv, Fab, V_H, Fd, Fabc, $F(ab')_2$, $F(ab)_2$ derived from A6.

The invention also teaches a library as described above, further comprising a plurality of libraries which are pooled, wherein at least a first and a second of said pooled libraries differ in the degree of biasing to wild-type amino acids.

The invention also teaches a library as described above, wherein said first and said second pooled libraries differ with respect to the degree of biasing of CDR3 region to produce fusion proteins with differing solubility characteristics.

The invention also teaches a library as described above, wherein said first and said second pooled libraries differ with respect to the degree of biasing to produce amino acid that are preferred for intermolecular interaction, said amino acids selected from a group including tyrosine, histidine, glutamine, asparagine, lysine, aspartic acids and glutamic acid.

The invention also teaches a method for creating a library of soluble proteins expressing heavy chain binding domains comprising generating a library of microorganism clones producing variant protein heavy chain binding domains by incorporating mutations into the binding subunit DNA of a non-camelid parental heavy chain binding domain in said microorganism clones.

The invention also teaches a method for creating a library expressing binding domains comprising:

(a) cloning a parental DNA sequence encoding a parental domain to create parental clones;

(b) replacing a variable region of said parental clones with a variant DNA sequence by adding by a series of step-wise in vitro syntheses variant nucleic acids to positions on said parental clone, said variant nucleic acids corresponding to positions of parental nucleic acids, to create a variant DNA sequence; and